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MUTATION IN THE GENUS ALTERNARIA

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Orda Allen Plunkett

ENTITLED Mutation in the Genus Alternaria

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Arts

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Mutation in the Genus Alternaria

I. Introduction

The present study arose from an interest which the writer had in the appearance of a sector of decidedly different color in a supposedly pure culture of Alternaria growing in a Petri dish on corn-meal agar. Single spores of this culture were grown and the colonies examined for further evidence of this phenomenon. It was found that quite often in these single-spore cultures variant sectors were formed, which retained the characteristic variation and remained different from the parent colony throughout many transfers. This led to a tentative assumption that these variant sectors represented mutants or saltants of more or less permanent nature, and a more detailed study of this phenomenon was therefore undertaken.

The writer has cultured on corn-meal agar, some 15 strains of Alternaria isolated from different hosts, to see if the phenomenon of variant sectors is equally common in all these species or races. These strains have all been grown from single spores to avoid the possibility of error arising from mixed cultures.

The variants found in the study of several strains have been carefully examined and compared in relation to each other

and to the original form.

II. The Present Status of the Mutation Question in Fungi and Bacteria

Bud variations, vegetative variations, sports, mutations and saltations have been known and studied among the higher plants for some time. In these cases such variations may or may not be connected with an intervening sexual act. That similar cases appear among bacteria and fungi is evident from a number of articles bearing on this subject published in the last few years.

The mutations occurring in bacteria have been divided by Dobell (16) into two classes, "those in which the change is functional (changes in the power of producing ferments or pigments) and those in which the change is structural". The greater number of mutations recorded for bacteria belong to the first class.

Massini (26) obtained from a case of enteritis an organism which at first produced whitish colonies on Endo's medium. After a time, however, red, daughter colonies appeared within the parent colony. Massini convinced himself, by carefully plating out the original culture, and by other means, that he had not been working with a mixed culture. The organism in the red daughter colonies had permanently acquired the power to ferment lactose and always produced red, and never white colonies. Even after transplanting to other media, free from lactose, they never lost this power. The typhosus-like original therefore, had given rise

to a number of new individuals which closely resembled the original, but which had acquired the power to ferment lactose. The white parts of the colonies containing the red, daughter colonies behaved exactly like the original when transplanted. The white race was therefore constantly undergoing a partial mutation into a pure red race.

Twort (42) recorded independently that certain coli-typhosus organisms were able to acquire the power to ferment certain sugars if grown in them for a sufficiently long time. By this method he was able to get a strain of B. typhosus which could ferment lactose and dulcite. The organism which had acquired this power retained it permanently, even after passage through a guinea-pig and cultivation in a dulcite-free medium.

Burke (10), Sauerbeck (33), Benecke (4) and Kowalenko (23) have isolated organisms similar to the one studied by Massini (26). Their results check his and in addition make an important addition to them, since they succeeded in testing individual organisms.

Kowalenko (23) found that a single individual, which at the outset was unable to split lactose, produced in the presence of this substance offspring, which were in part like itself, and in part able to split lactose. The only objection to Massini's results was therefore removed. Single individuals from white colonies gave rise to mutating races; single individuals from "red" colonies retained their characters in following generations.

Their properties were unaltered by passage through animals, by changes of temperature, or by phenol or other drugs.

Muller (27) demonstrated that all typical races of B. typhosus are unable to ferment rhamnose. When grown in a medium containing this sugar, however, the colonies develop daughter colonies (nodules) consisting of individuals which have permanently acquired the power of splitting rhamnose. A single non-rhamnose splitting individual gives rise in the presence of this substance to offspring which are partly like itself and partly able to ferment rhamnose. Mutations of this sort invariably occur from single individual cultures and under no conditions do the rhamnose splitting individuals lose their power.

Schröter and Gütjahr (35) record the following observations. A race Y of coli typhosus-group organisms cannot ferment maltose. After cultivation in a medium containing this sugar, however, it partially acquires the power, thus coming to resemble Flexner's bacilli. They found similar changes in related organisms; for example, Shiga-Kruse bacilli may acquire the power of splitting both maltose and saccharose when grown in media containing these sugars. The change appears to be permanent, for once acquired by the race the property is never lost.

Sobernheim and Seligmann (36) have isolated an organism which behaves exactly like Massini's B. coli mutabile. In addition they record that they have obtained four different pure races from one pure original race grown from a single individual. From

a coli-typhosus-group organism they have obtained in pure culture (1) a true Gartner strain, (2) a similar strain, but differing in agglutinating power, (3) a typical typhosus, and (4) a strain almost identical with B. coli mutabile (Massini).

The papers of Burri and Duggeli (13) and Burri and Andrejew (12) may be considered as parts of Burri's admirable paper of 1910 (11). Burri (11) has isolated a race of organisms of the coli-typhosus group which are unable to ferment saccharose and lactose. He calls this race Bacterium imperfectum. It never acquired the power of splitting lactose, but on the other hand, when grown in media containing saccharose, some colonies acquired the power of splitting this sugar.

Burri's (11) observations were originally made upon organisms grown in "shake cultures" and not on Endo's medium. The cultures were made in the ordinary way and also from single individuals isolated by his Indian ink method. Each non-saccharose splitting mutating organism was found to give rise to only a very low percentage of saccharose-splitting colonies. The latter, however, had acquired the power permanently, and always retained this character in following generations, even after any number of subcultures in many different media.

By a number of ingenious experiments Burri (11) attempted to determine what percentage of the offspring of B. imperfectum underwent mutation into B. perfectum. He concludes that all of the individuals of the imperfectum race are able, under suitable

cultural and environmental conditions, to mutate into perfectum forms.

In another set of experiments he has shown that the change from the imperfectum to the perfectum type is not a sudden change but a rather gradual one. Between the non-saccharose-splitting imperfectum and the saccharose-splitting perfectum intervene many generations of individuals showing every transitional stage. This power once acquired by a race is never lost.

Burri (11) supposes that the power to ferment saccharose is latent in every B. imperfectum individual, probably in the form of a zymogen or pro-ferment of some sort. The enzym is produced gradually by the constant action of the sugar on successive generations. He thinks that the newly acquired power of attacking saccharose does not represent a regeneration of a power originally present in the race but temporarily lost.

Bernhardt and Markoff (5) obtained a coli-typhosus-like organism which was capable of fermenting lactose, but in which, after culturing for some time, and passage of the organism through mice and rabbits, the power of fermenting lactose was lost. Thus in this case it would seem that a lactose-fermenting organism which usually remains constant has reverted, or lost this power.

Barethlein (3) found a very similar case to that above cited in several races of the coli-typhosus group: viz that many of the lactose-splitting organisms, when grown for some time on ordinary media, lost the power of fermenting lactose. His results

seem to disagree with those of most other writers. The reason for this may be that he was dealing with different races of apparently the same organism which do not all behave alike.

Revis (30) in studying certain organisms of the coli-typhosus group which produce both acid and gas when grown on peptone broth containing lactose, was gradually able to acclimatize these organisms to grow in a medium containing 0.1 per cent malachite green. These organisms completely lost their power to form gas in the original medium although they still produced acid. The dye appears therefore to have made a lasting change in their method of attacking certain food substances.

Wolf (45) carried out a number of extensive experiments to induce mutation in pigment production. He found that the same chemical substance often produced several kinds of mutations. Thus mercuric chloride produced a dark red race and a pure white race in B. prodidgiosus. Here the mutation seems to have been in opposite directions; in one case the intensifying of color, and in the other the loss of color. Similarly cadmium-nitrate produced a constant dark red mutant and a white reverting strain which did not remain constant in following generations.

Eisenberg (17) by cultivating Bacillus anthracis on glycerin-agar obtained a race of organisms which did not produce spores and which remained constant in this character.

Barber (2) in studying Bacillus coli found that there

were certain long individuals, longer than the ordinary B. coli, constantly present in his cultures. By isolating and growing these individuals he found that the greater number of them produced colonies of individuals typically like B. coli; however, in a single instance he obtained one which retained the character and gave only long individuals. It seems from his results that the long individuals were of two kinds, though outwardly more or less alike. The majority represented merely temporary modifications which did not produce like individuals in the following generations while only a few were permanently mutated organisms.

Revis (31) maintains that by growing B. coli on peptone broth to which malachite green had been added, he produced a strain which was structurally different, as well as morphologically different, from the original culture. His experiments were not carried on by isolation of single individuals, and although the purity of the culture is guaranteed his work is open to some criticism and should be repeated using single individuals.

Brierley (8) has divided the previous records of mutations in fungi into two groups. "In the first are those changes in the genetic constitution which appear to bear a direct and purposive relation to certain conditions which have operated during the development of the organism or that of the preceding generation. The second category of mutations include those cases in which the genetic change is apparently quite fortuitous, and this group is divisible into two sub-groups. In the first of these

the change is associated with certain conditions which interfere with the normal metabolic reactions of the organism. For example, by treating the organism with toxic substances, or by subjecting it to extremes of temperature or dessication, various intermediate morphological or physiological changes are induced, such as alterations in spore dimensions or coloration, or growth on standard media. In the second sub-group the changes occur spontaneously under, so far as may be judged, perfectly normal conditions of development." The latter sub-group would include the various mutant forms of Alternaria discussed in the present paper.

The best known case of purposive mutation, perhaps, is that discussed by Massee (25). In this case Massee sowed spores of Trichothecium candidum on leaves injected with a sugar solution and from the growth thus secured inoculated other injected leaves. This was done for several generations of the fungus, after which spores from the last growth were sown on normal leaves and infection obtained. "This means that after 12 generations of the fungus educated to grow in Begonia by means of a chemotrophic substance (a solution of cane sugar) the faculty of parasitism had been acquired for this particular host plant. --- By similar means a parasitic fungus can be induced to become parasitic on a new host."

Similar to the findings of Massee (25) are those described by Salmon (32) on the adaptive parasitism of Erysiphe graminis by means of growth upon bridging hosts, or the parallel results of Marshall Ward (43), Freeman (20), Freeman and Johnson (21),

Johnson (22), Pole Evans (19) and others, obtained with various rust fungi. None of the above work, however, was carried on with single-spore strains of the organism in question and is therefore open to criticism. The work of the latter writers has been contravened by the work of Reed (29), Stakman (37), Stakman and Piemeisel (38), Stakman, Parker and Piemeisel (39), and Stakman, Piemeisel and Levine (40). As a result of their investigations the latter state; "The results of the experiments with P. graminis tritici-compacti show that barley which both theoretically and from the results obtained by previous investigators might be expected to increase the infection range does not do so. Even susceptible varieties of wheat do not change the parasitic capabilities of the rust so as to enable it to attack a normally resistant variety. Furthermore the rust does not acquire additional virulence when associated for a long time with a given host. --- Although it is possible that rusts may change and new biologic forms may develop, it seems more probable that the change is either a very gradual one, extending over long periods of time, or that they change by mutation. No evidence of mutation, however, was obtained in the present investigation. The difference may be one of evolution as compared with experimentally induced change. For practical purposes, however, it seems perfectly safe to say that no certain and marked changes in biologic forms need be expected as a result of growing on bridging hosts; nor does it seem probable that biologic forms are able to gradually adapt themselves to semi-

congenial hosts by constant association with those hosts. The writers unsuccessfully tried to get evidence of such adaptation."

The records of instances of mutation in fungi induced by exposure to unfavorable conditions are few in number. Arcichovskij (1) obtained a form of Aspergillus niger with yellow-brown spores, which rose in a pure culture of the black form growing in Raulin's fluid to which 0.0001 per cent of zinc sulphate had been added. This form produced spores more quickly and was of faster growth than the original culture. He mentions that he has carried the form through a number of generations, but does not make clear, whether it was grown on a medium containing zinc, or on a normal medium.

Schiemann (34) obtained various mutants of Aspergillus niger from a single-spore culture by growth on media containing potassium bichromate, and by exposure to extreme temperatures. The potassium bichromate was added in a concentration of 1 to 2,000 and 1 to 20,000 parts to a medium prepared from cane-sugar, peptone, potassium nitrate, magnesium sulphate and potassium di-hydrogen phosphate. From the first generation of a culture on a medium containing potassium bichromate, 1 to 2,000, a brown form was isolated and cultured on malt-agar. This mutant remained constant through 32 generations. From the 11th generation of a culture growing on a medium containing potassium bichromate, 1 to 20,000, a form was isolated which varied from a dirty white to a bright-brown in color. This mutant remained constant through 23 genera-

tions on malt-agar. From a culture growing on malt-agar slants free from any poisonous substance, but exposed to a maximum temperature of 44° - 45° C., a deviating growth was produced. The colony of this form was of faster growth than the original. It had a loose mycelium with many aerial hyphae and very long conidiophores; (3-4 mm. as compared to 1-2 mm. of the original). This mutant remained constant through 17 generations when cultured on malt-agar at normal temperature.

The brown mutant appeared three times; twice in the medium containing potassium bichromate and once in a culture which had neither been exposed to high temperature nor contained potassium bichromate. Thus it seems that mutation occurs both with and without these unfavorable conditions for growth.

Waterman (44) by cultivating Penicillium glaucum, of a supposedly pure race, in the presence of p-oxybenzoic acid, protocatechetic acid, salicylic acid and trichloracrylic acid was able to induce mutation. The mutant thus produced was distinctly lighter in color, when grown on malt-gelatin, in contrast to the original culture. It differed also from the primitive form in having fewer spores, a decidedly different odor, as well as a slower rate of growth. In continued cultivation on malt-agar the mutant, which in all cases seemed the same, remained constant.

He also found that a pure culture of Aspergillus niger when grown for some time in a 2 per cent solution of galactose gave three distinct forms; namely, the original black form, a

brown form and a white one. The brown and white forms when isolated on malt-agar gave fewer spores than the original, and the white form fewer than the brown one. There was also a decrease in the color intensity of the spores in the mutants, the spores being brown instead of black. There was also a difference in the metabolism of the original and the mutants, the latter showing a more vigorous combustion of p-oxybenzoic acid to carbonic acid. In this respect the white form had a more vigorous combustion than the brown one.

Brierley (8) reports that he has tried carefully to repeat the above experiments of Arcichovskij (1), Schiemann (34) and Waterman (34), using single-spore cultures of Aspergillus niger and Penicillium italicum. He was unable to do so and states, "Although modifications in the color of the spores, general morphological facies, and physiological reactions were observed, these affected the whole growth acted upon to an equal degree; but the reproductive bodies of the modified fungi, gave in every case the original result. These changes were phenotypic and not genotypic and throughout the whole series of experiments not a single mutation occurred."

Stevens (41) tested the effect of the amount of the medium, humidity, temperature extremes, and content of the medium upon cultures of Helminthosporium. He found that, although there were variations produced by these environmental changes, they were mere modifications and did not remain constant when the fungus was

returned to favorable environmental conditions.

Crabill (14) attempted in four ways to induce mutation artificially in Coniothyrium pirinum but without success.

The literature dealing with the general question of mutation in the Eumycetes has been fully discussed by Stevens (41), and it is not deemed necessary to go into a detailed review of these papers here. There are, however, a few which he has not mentioned and some which have a more or less direct bearing upon the present problem which deserve some consideration.

Crabill (14) working with single-spore cultures of Coniothyrium pirinum described two strains which he designated as plus and minus. These strains differed markedly in several characters, particularly in size and abundance of pycnidia, and in color of colony. He found in his different strains variations ranging from many, large, fully developed pycnidia to few small pycnidia, verging on complete sterility. He says; "The cultural studies show that minus strains may rise from plus strains by sudden sporting or mutation. An objection might be raised that these cultures were impure, i.e. mixtures of two strains. In anticipation of such an idea it seems desirable to state that frequent pourings of dilution cultures were used to preclude such a possibility. Progeny were then selected only from well-isolated plants, microscopic examination of which showed that each had been derived from a single spore.--- Both strains have repeatedly arisen from the progeny of a single plus spore. When once purified the minus strain

remains constant from generation to generation. The variation is apparently occurring in only one direction. --- The only explanation which remains is that the minus strain is a sport or mutant arising from the plus strain at irregular and unprognosticable intervals."

The plus strains by sudden variation give rise to the minus strains but the minus strains were not seen to give rise to the plus strains. Contrary to the findings of Stevens (41) and myself he states that the variation apparently occurs in the spores and not in the mycelium. He pictures sectoring and colony differences quite similar to those shown by Stevens (41) for Helminthosporium and those pictured in this paper for Alternaria.

Burger (9) in studying Colletotrichum gloeosporioides found variant sectors occurring in certain of his cultures. Upon transferring mycelium from these sectors he obtained a strain distinctly different from the original in morphological cultural characters. Since the sectoring was of common occurrence in his cultures he thought of the possibility of mixed cultures and grew colonies from single spores. He found that the sectoring continued and states; "One might be led to conclude from the foregoing data that Colletotrichum gloeosporioides is constantly giving off new types under natural conditions, as well as in artificial cultures.

Brierley (8) has reported an albino mutant of Botrytis cinerea. This strain developed by forming a colorless sclerotium

in a culture which normally formed black sclerotia. The parent colony originated from a single spore and had been under cultivation for some time. Brierley states in summary: "The colorless form arose spontaneously without any evident relation to external conditions or stimuli, in a single sclerotium of a culture which on accepted criteria was a pure line. The new form is apparently differentiated from its parent in respect to a single character only, that of color, and this albinism would appear to be associated with the absence of cromogen. The change has occurred once only, and has given rise to a form unknown in nature and perfectly constant under all conditions. It would seem possible to place only one interpretation on these facts, that of mutation, and accordingly when I was privileged to exhibit this form to the Linnean Society of London on April 3, 1919, I described it as 'an albino mutant of Botrytis cinerea.'"

Blakeslee (6) reports numerous variations in single-spore cultures of Mucor. Many of these mutants tend to revert to the original form when cultured; however, he has found two mutants which are more stable and maintain the varying characteristics.

Stevens (41) while studying a species of Helminthosporium causing a foot-rot of wheat noticed the prevalence of sectors of different color and growth occurring in his cultures. Single-spore cultures of the original culture were made and carefully studied. He found that saltation occurred quite frequently in this species. The saltants differed from the original strain in

a number of ways, particularly however, in color of colony, rate of growth, number of spore septations and length of spores. The case he describes for Helminthosporium and the pictures and plates presented are quite similar to those presented for this paper.

There exists among the various writers on the mutation question differences as to the usage of the term mutation. The bacteriologists denote (Dobell (16)) " a permanent change - however small it may be - which takes place in a bacterium and is then transmitted to subsequent generations. All other changes which are impermanent depending generally upon changes of the environment - and hereditarily fixed, are called modifications." Brierley (7) defines mutation as "a genotypic change in a pure line". In the present paper the term mutation is used to denote a sudden morphological change which is transmittable to subsequent generations. In this respect it does not necessarily imply a nuclear change, and is similar to the terms saltation as used by Stevens (41), or mutation as used by Blakeslee (6), Crabill (14) and Burger (9).

From the above summaries of the mutation question in bacteria and fungi one may draw certain conclusions. If we consider that the work of the above writers has been carefully done, then we must conclude that mutations occur under cultural conditions and it is reasonable to suppose that such phenomena occur in nature. Many of the above papers are undoubtedly open to criticism, especially those dealing with bacteria, because of the loose methods

employed or conclusions drawn from too scanty evidence. However, in such work as that of Burri (11) and Barber (2), both of whom worked with single-spore cultures and carried on their work under the best of conditions it can hardly be denied that mutation has taken place.

Brierley (7) is not inclined to accept the recorded mutations for bacteria on the grounds that little or nothing is known of the genetic constitution of bacteria, and also since the strains in question were not derived from pure lines. He looks upon the variations recorded as being mere segregations of heterogynous strains and not mutations in his sense of the term.

A possible method by which these strains might be of mixed genetic constitution has been brought out by the work of Löhnis and Smith (24) on the existence of life cycles in bacteria. They state: "The life cycle of each species studied is composed of several sub-cycles showing wide morphological and physiological differences. They are connected to each other by the symplastic stage. Direct changes from one sub-cycle into another occur, but they are rather rare exceptions." In this respect it is not inconceivable that in the bacterial life cycle there may exist some phase more or less comparable with the sexual fusions of other organisms. Löhnis and Smith (24), for example note a symplastic stage in which, "the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of the cell walls, as well as cell content, or by

the melting together of the contents of many cells which leave their empty cell walls behind them". Furthermore they state, " another mode of interaction between the plasmic substance in bacterial cells was observed consisting in the direct union of two or more individual cells. This conjunction seems to be of no less general occurrence than the process first mentioned". Such conditions as these, if existing in the bacteria may offer an explanation of the apparent mutations in bacteria.

The suggestion has been offered that the mutations and saltations of Crabill (14), Burger (9), Stevens (41) were due to segregation in a heterozygous strain. It is true that in all these cases cultural work was begun from a single asexual spore which to all external appearances was homozygous. Yet it is true for some, and possibly true for all, that an ascigerous or sexual stage exists. It is also conceivable that, if the spores from which these cultures originated had been derived in the not far distant past from an ascigerous stage, the possibility of their being heterozygous exists. Stevens (41) states, "this possibility is purely hypothetical but it appears to offer a possible explanation for the saltations in Helminthosporium". Dastur (15) found variation in Gloeosporium piperatum a common occurrence only in strains recently derived from perithecia. This fact would tend to support such an hypothesis.

It may be that some of my cultures of Alternaria which are mutating have been recently through a sexual stage, while

others which show no signs of mutation have not. All of my cultures have been secured from asexual spores and none of them have shown signs of producing perithecia. One point against such an argument is the fact that my culture No. 1 which has been under constant cultivation for over 14 months and during that time has been grown three times from single spores still continues to mutate.

Brierley (8) offers entirely different suggestions as to the possible explanation of his mutation in Botrytis cinerea, in which sexuality is unknown. He suggests the possibility of a nuclear transference due to anastomosis of the mycelia, or the cytoplasmic contamination by such anastomosis. He presents no evidence for his suggestions, and although anastomosis is common among fungi it would take detailed cytological study to prove a nuclear transference or fusion.

III. Source of Materials

The strains or species of Alternaria used in these studies have been secured from widely different and varied sources. Strain 1 was obtained from germinating millet purchased from a Champaign seed-house. Strain 7 was isolated from a ripe wheat-head from Granite City, Illinois. Strain 10 was secured from dead onion seed-stalks from Crawfordsville, Indiana. Strain 15 was isolated from decaying apples obtained from a grocery in Urbana. Other strains which have failed to show signs of mutation have been isolated and studied from, leaf-spots on Datura, Lilium and

Yucca, from fruits of okra, morning glory, snow berry, mango and pepper, and from dead blades of grass and corn.

IV. Methods Employed for Isolation and Growth

Cultures of Alternaria from the different sources were usually obtained by planting infected tissue of the host on agar, or by scraping spores from the host into a few drops of sterile water which was then added to a tube of agar and poured into a sterile Petri-dish. The growth thus secured was examined and transferred until, as far as could be ascertained, a pure culture had been obtained. Single-spore cultures were then made in order to be sure that the cultures were pure. This was accomplished in the following manner. Spores from the cultures were transferred to a tube containing about 10 cubic centimeters of sterile water. It was quite essential that only a few spores be placed in the water. In order to ascertain the approximate number of spores per oese in the suspension, two oeses were transferred to a slide and examined under the microscope. If the number of spores thus obtained was under 12 the suspension was considered satisfactory. Two oeses of the suspension were then transferred to a tube of melted agar at 42° C., thoroughly mixed by rolling between the hands, and poured into a sterile Petri-dish. As soon as the agar had hardened the poured plates were examined by inverting them under the low power of the microscope. In this way single spores, well isolated from other spores, were located and their position marked by drawing a circle around them with Indian ink, on the bottom of the

Petri-dish. Great care was used in making sure that only one spore was inside the circle. The agar within the circle was carefully lifted out, transferred to another sterile agar plate and again examined to make sure that only one spore had been transferred. The single spore was then allowed to germinate and produce a colony.

Transfers from the colonies originating from single spores have been made to plates of corn-meal agar and grown at room temperature to observe whether mutation would take place or not. In all the cultural work corn-meal agar, made by the following formulae, has been used.

Fifty grams of corn-meal were added to 1,000 cc. of distilled water and cooked for one hour over a water bath at 60° C., then filtered through cloth and filter paper and 13 grams of agar shreds added. The above medium was then cooked for one and one-half hours over a water bath, filtered through cotton, tubed and sterilized in the autoclav for 20 minutes at fifteen pounds pressure.

Care has been taken to have the media as nearly constant as possible to avoid variations due to the quality of the medium.

Appearance of Mutations

The mutations herein reported always arose as sectors differing in color from that part of the parent colony adjacent to them. Transfers were made from these variant sectors to poured sterile agar-plates, and a transfer of the normal colony placed in the same plate some distance from the variant transfer. The variant transfer was marked as M and the normal transfer designated as O.

The colonies produced from such transfers were allowed to grow until they filled the plate when other transfers were made, and so on for a large number of generations. By this method it was possible to study the mutant and the original under approximately the same conditions. The two colonies grown in this way differed most markedly in color, presence or absence of aerial mycelium, and in conidial production. In practically all cases such mutants arising from distinct sectors have remained constant, though occasionally one is obtained which in turn gives off sectors which are apparently reversions to the original form. In the contrast studies of the mutants and the originals care has been taken that the thickness of the medium be the same all over the place, since it was found that the thickness of the medium plays an important part in the appearance of the colony.

Throughout the paper M denotes a mutant while O denotes the original culture. I have given the different strains of Alternaria tested for mutation a serial number. Thus O 10, M2 refers to a mutant, No. 2, arising from original culture No. 10. A generation is taken to mean the growth produced on a sterile plate by a transfer of mycelium from another culture.

In original culture No. 10 mutant 1 appeared in the 2nd generation. This culture has been giving rise to mutants for more than 14 months. Some 18 of these mutants have been isolated and studied but they appear to be the same, or very closely alike, and are similar to mutant 1. Mutant 15 appeared in the 22nd generation of the original culture and in the 4th generation after re-

isolation from a single spore.

In culture No. 7 mutant 1 appeared in the 12th generation. This mutant has appeared but once and has repeatedly given off sectors which appear to be reversions to the original, since no significant differences are brought out by contrast studies.

In culture No. 10 two distinct mutants have been isolated from among the many variant sectors given off by the original culture. Mutant 1 appeared in the 4th generation of the original culture; mutant 2 in the 9th generation. Other mutants have arisen from this culture but in contrast studies have not appeared to be distinct from mutant 1. Thus it might be said that mutant 1 has appeared several times. Mutant 2 has arisen but once and has remained constant.

Modifications

Throughout these cultural studies certain modifications have arisen. The most common modifications are in the form of small sectors of different color at the very edge of any old colony which is drying out. In most cases transfers from these small sectors give normal colonies like the original. A form of modification less common than the above is the appearance of light or dark radiating streaks in original cultures Nos. 7 and 10. Transfers made from these streaks produce normal colonies. In a normal colony which has grown to the edge of the plate white aerial mycelium is often produced on the sides of the plate. This upon transfer does not maintain its characteristic white appearance but gives

a normal colony. In cases where the medium is not the same thickness throughout the plate certain modifications are produced which do not maintain themselves when transferred. Modifications have been induced by the addition of certain chemicals and toxic substances to the medium. Transfers from such modifications, grown on a normal medium, produce normal colonies.

V. Methods Employed for Contrast Studies of Mutants and Originals

In the following studies for the contrast in color of colonies, zonation of colonies, rate of growth, presence or absence of aerial mycelium and abundance of conidial production, the mutants have been grown side by side with each other and with their parent, under practically the same conditions. For the contrast of length and breadth, conidia were measured under the microscope by means of an ocular micrometer having a value of 3.4 mic. per space. A mechanical stage was used to move the slide on the microscope. All conidia were measured which came between certain lines on the ocular micrometer. In this way unconscious selection of conidia was avoided. The conidia were divided into classes and plotted on cross section paper according to the number of ocular spaces occupied. In cases where conidia fell midway between spaces note was made of them and they were equally divided between the two classes. The results of these measurements and the results of counting the number of cross and longitudinal septations have been plotted in the form of polygons and curves and compared biometric-

ally. In contrasting the color of the conidia, mycelia and media, two Leitz microscopes of approximately the same magnification were used. The fields of these two microscopes containing the desired material were brought together by using and E. Leitz, Wetzlar comparison ocular. In this way the different shades and tints could be quite accurately compared.

In view of the fact that much variation is caused by the quality of the medium, (Planchon (28)) the media used in all cultures has been made as nearly constant as possible. To avoid differences due to the age of the cultures, colonies of the same age have been used in all contrast studies. By this method it has been possible to add some significance to the differences in color of spores and mycelium, which would not be justifiable if cultures of unequal age had been used.

Color of Colonies on Agar

Culture No. 1.

The original culture gives a very light grey colony with white aerial mycelium. Mutant 1 has a reddish-brown colony. Mutant 15 gives a gray colony, but much darker in hue than the original, and it has dark aerial mycelium.

Culture No. 7.

The original colony of this culture is lead colored. Mutant 1 is light-brown with a bluish tint. The mutant is much darker than the original but has not nearly as much brown color as mutant 1 of culture No. 10, or mutant 1 of culture No. 15.

Culture No. 10.

The original culture has a colony that is dark gray approaching black. Mutant 1 has a reddish-brown colony much lighter in color than the original. Mutant 2 gives a colony similar in general color to the original but of brown tint. It is of much darker hue than mutant 1 but does not have the reddish-brown cast of mutant 1.

Culture No. 15.

The original culture gives a bluish-gray colony; mutant 1 a reddish-brown colony similar in appearance to mutant 1 of culture No. 1, but lighter in hue than mutant 1 of culture No. 7 and mutant 1 of culture No. 10.

Zonation of Colonies

Culture No. 1.

There is no zonation in the colonies of the original; a slight zonation in the colonies of mutant 1 and a very distinct zonation in the colonies of mutant 15.

Culture No. 7.

Colonies of the original show a slight zonation and light streaks radiating from the center. Colonies of mutant 1 show a distinct zonation which is more prominent than in the original. The radiating streaks present in the original are absent in the mutant.

Culture No. 10.

Colonies of the original culture show no marked zonation.

Colonies of mutant 1 show a distinct and marked zonation, while those of mutant 2 show a slight zonation which is not so pronounced as that of mutant 1.

Culture No. 15.

Colonies of the original culture show very slight zonation while those of mutant 1 show distinct zonation.

Rate of Growth

Contrast studies of the different mutants and their originals have shown little or no difference in their rate of growth. There appears to be a slight difference between original 15 and its mutant 1 in that the mutant grows slightly faster than the original. The difference here is very slight and not readily apparent.

Color of Mycelium

Culture No. 1.

The original culture has a hyaline mycelium. The mycelium of mutant 1 is yellowish-brown. The mycelium of mutant 15 is gray being much darker than that of the original.

Culture No. 7.

The mycelium of mutant 1 is thicker and slightly darker than that of the original culture.

Culture No. 10.

The mycelium of the original is darker than that of either of the mutants. The original culture has a dark-gray mycelium; mutant 1 a nearly hyaline mycelium with a yellowish cast; mu-

tant 2 a light-gray mycelium.

Culture No. 15.

The mycelium of the original culture is lead-colored while that of mutant 1 is much lighter with a yellowish cast.

Color of Medium.

In cultures Nos. 1, 7, and 15 neither the originals nor the mutants color the medium in which they are growing. In culture No. 10 the medium is not colored by the original culture or mutant 2, but is colored slightly yellow by mutant 1.

Aerial Mycelium

Culture No. 1.

There is an abundance of aerial mycelium produced by the colonies of the original culture. The colonies of mutant 1 do not produce aerial mycelium; those of mutant 15 produce aerial mycelium, but not so abundantly as colonies of the original. The mycelium of mutant 15 is quite flocculose, a character entirely absent in the original culture and in mutant 1.

Culture No. 7.

There is an abundance of aerial mycelium produced by the original culture, together with numerous white tufts. Mutant 1 produces considerably less aerial mycelium, and the white tufts are entirely lacking.

Culture No. 10.

The original culture produces an abundance of aerial mycelium; this is lacking in mutant 1 and is produced in less abundance in mutant 2 than in the original. The mycelium of mu-

tant 2 is quite flocculose, a character lacking in the original and in mutant 1.

Culture No. 15.

The original culture produces abundant, long, aerial mycelium. Mutant 1 produces some aerial mycelium which is short and with age comes to lie flat on the suface, producing a felt-like weft.

The following mathematical calculations represent the mean (M), standard deviation (σ) and coefficient of variability (C.V.) as obtained by the formulae of Davenport and Rietz in Illinois Agr. Exp. Sta. Bul. 119, for the graphs listed on plates 1 to 9; representing conidial measurements of length and width and the number of cross and longitudinal septa. On accepted criteria in comparison of the means of graphs by this method it is assumed that if the difference between the means of two graphs is greater than 6 times the probable error, the chance is 19260 to 1 that the two strains are different. It has not been thought best to consider differences of less than 6 times the probable error to be significant.

Length of Conidia

Culture No. 1.

Original. Plate 1. Graph No. 1A.			Mutant 1. Plate 1. Graph No. 1B.			Mutant 15. Plate 1. Graph No. 1C.		
M.	8.73	.08	M.	7.38	.05	M.	7.50	.08
σ.	1.72	.05	σ.	1.19	.04	σ.	1.88	.06
C.V.	19.76	.69	C.V.	16.17	.55	C.V.	25.13	.91

The difference between the means of graphs 1A and 1B is 17 times the probable error. The difference between the means

of graphs 1A and 1C is 15 times the probable error; that of graphs 1B and 1C only 1.5 times the probable error. The graphs show that there is a wide difference in conidial length between the original and the mutants, but that there is only a slight difference in the conidial lengths of the mutants.

Culture No. 7.

Original. Plate 1. Graph No. 5A.			Mutant 1. Plate 1. Graph No. 5B.		
M.	6.77	.07	M.	7.17	.08
σ.	1.48	.05	σ.	1.84	.06
C.V.	21.91	.77	C.V.	25.75	.92

The difference in the means here is only 5 times the probable error which shows no very significant difference in the conidial lengths of the original and the mutant.

Culture No. 10.

Original. Plate 1. Graph No. 9A.			Mutant 1. Plate 1. Graph No. 9B.			Mutant 2. Plate 1. Graph No. 9C.		
M.	8.84	.13	M.	8.03	.11	M.	10.07	.12
σ.	2.90	.09	σ.	2.44	.08	σ.	2.60	.08
C.V.	32.80	1.21	C.V.	30.20	1.09	C.V.	25.84	.92

By comparison of the means of graphs 9A and 9B we find the difference to be 6 times the probable error; of graphs 9B and 9C, 15 times the probable error. The graphs thus show that there is a wide difference between the original and the mutants, and between the mutants themselves, in respect to conidial length.

Culture No. 15.

Original. Plate 1. Graph No. 13A.			Mutant 1. Plate 1. Graph No. 13B.		
M.	6.10	.05	M.	7.44	.07
σ.	1.16	.03	σ.	1.48	.05
C.V.	19.10	.66	C.V.	19.96	.62

The difference between the means of graphs 13A and 13B is 19 times the probable error showing a wide difference between the length of the conidia of the original and the mutant.

Width of Conidia

Culture No. 1.

Original. Plate 2. Graph No. 2A.			Mutant 1. Plate 2. Graph No. 2B.			Mutant 15. Plate 2. Graph No. 2C.		
M.	3.32	.03	M.	3.23	.02	M.	3.20	.02
σ	.71	.02	σ	.54	.01	σ	.50	.01
C.V.	21.46	.75	C.V.	16.87	.58	C.V.	15.93	.55

The difference between the means of graphs 2A and 2B is 3 times the probable error; of graphs 2A and 2C, 4 times the probable error; of graphs 2B and 2C no difference. It is concluded from the above evidence that there is no essential difference between the original and the mutants, or between the mutants themselves, in regard to conidial width.

Culture No. 7.

Original. Plate 5. Graph No. 6A.			Mutant 1. Plate 5. Graph No. 6B.		
M.	2.95	.07	M.	3.03	.03
σ	.49	.01	σ	.70	.02
C.V.	16.81	.58	C.V.	23.31	.82

The difference between the means of graphs 6A and 6B is only 3 times the probable error and is therefore not significant.

Culture No. 10.

Original. Plate 6. Graph No. 10A.			Mutant 1. Plate 6. Graph No. 10B.			Mutant 2. Plate 6. Graph No. 10C.		
M.	3.12	.03	M.	3.26	.03	M.	2.89	.02
σ	.66	.01	σ	.75	.02	σ	.55	.01
C.V.	20.56	.72	C.V.	23.19	.82	C.V.	19.20	.67

The difference between the means of graphs 10A and 10B is equal to 3 times the probable error; that of graphs 10A and 10C, 9.5 times the probable error, and that of graphs 10B and 10C, 12 times the probable error. This shows that there is only a slight difference between the original and mutant 1 in the width of conidia, but a distinct difference between the original and mutant 2, and between mutants 1 and 2 in this respect.

Culture No. 15.

Original. Plate 9. Graph No. 14A.			Mutant 1. Plate 9. Graph No. 14B.		
M.	3.01	.03	M.	3.17	.02
σ.	.63	.02	σ.	.58	.01
C.V.	21.10	.74	C.V.	18.48	.64

The difference between the means of graphs 14A and 14B is only 5 times the probable error. Although this is quite a large difference it is not considered sufficient to be significant.

Cross Septa

Culture No. 1.

Original. Plate 3. Graph No. 3A.			Mutant 1. Plate 3. Graph No. 3B.			Mutant 15. Plate 3. Graph No. 3C.		
M.	3.24	.03	M.	2.88	.03	M.	3.08	.04
σ.	.74	.02	σ.	.75	.02	σ.	.87	.02
C.V.	22.98	.81	C.V.	26.25	.94	C.V.	28.55	1.03

The difference between the means of graphs 3A and 3B is 12 times the probable error; of graphs 3A and 3C, 5 times the probable error, and between graphs 3B and 3C, 6 times the probable error. This shows that there is a distinct difference between the original and mutant 1, and between mutant 1 and mutant 15,

but that there is no distinct difference between the original and mutant 15 in regard to the number of cross septa.

Culture No. 7.

Original. Plate 5. Graph No. 7A.			Mutant 1. Plate 5. Graph No. 7B.		
M.	2.76	.03	M.	2.63	.04
σ.	.71	.02	σ.	.86	.02
C.V.	25.95	.93	C.V.	32.68	1.21

The difference between the means of the above graphs is only 3 times the probable error and therefore does not show a distinct difference between the original and the mutant in number of cross septa.

Culture No. 10.

Original. Plate 7. Graph No. 11A.			Mutant 1. Plate 7. Graph 11B.			Mutant 2. Plate 7. Graph 11 C.		
M.	3.39	.06	M.	3.20	.05	M.	3.86	.05
σ.	1.32	.04	σ.	1.07	.03	σ.	1.18	.03
C.V.	38.90	1.52	C.V.	33.56	1.27	C.V.	30.57	1.12

The difference between the means of graphs 11A and 11B is 3 times the probable error; of graphs 11A and 11C, 8 times the probable error, and of graphs 11B and 11C, 11 times the probable error. This shows that there is only a slight difference between the number of cross septa in the original and mutant 1; a decided difference between the number in the original and mutant 2, and between the number in mutant 1 and mutant 2.

Culture No. 15.

Original. Plate 9. Graph No. 15A.			Mutant 1. Plate 9. Graph No. 15B.		
M.	2.65	.03	M.	3.06	.03
σ.	.81	.02	σ.	.64	.02
C.V.	30.85	1.06	C.V.	21.08	.74

The difference between the means of graphs 15A and 15B is 13.5 times the probable error showing that there is a wide and distinct difference in the number of cross septa in the original and the mutant.

Longitudinal Septa

Culture No. 1.

Original. Plate 4. Graph No. 4A.			Mutant 1. Plate 4. Graph No. 4B.			Mutant 15. Plate 4. Graph No. 4C.		
M.	1.68	.05	M.	1.34	.03	M.	1.66	.05
σ.	1.06	.03	σ.	.81	.02	σ.	1.05	.03
C.V.	63.48	2.87	C.V.	60.82	2.70	C.V.	63.35	2.86

The difference between the graphs 4A and 4B is 7 times the probable error; of graphs 4A and 4C it is within the error; of graphs 4B and 4C it is 6 times the probable error. This comparison shows that there is a difference between the original and mutant 1 in the number of longitudinal septa; no difference in this respect between the original and mutant 15, and a wide difference between mutant 1 and mutant 15.

Culture No. 7.

Original. Plate 5. Graph No. 8A.			Mutant 1. Plate 5. Graph No. 8B.		
M.	0.62	.03	M.	0.91	.04
σ.	0.76	.02	σ.	0.85	.02
C.V.	12.23	.41	C.V.	9.40	.31

The difference between the means of graphs 8A and 8B is 7 times the probable error which shows a difference between the original and the mutant in respect to the number of longitudinal septa in the conidia.

Culture No. 10.

Original. Plate 8. Graph No. 12A.			Mutant 1. Plate 8. Graph No. 12B.			Mutant 2. Plate 8. Graph No. 12C.		
M.	0.77	.04	M.	1.46	.05	M.	0.87	.04
σ.	0.97	.03	σ.	1.18	.03	σ.	0.91	.03
C.V.	12.50	.43	C.V.	8.07	.40	C.V.	10.55	.35

The difference between the means of the graphs 12A and 12B is 17 times the probable error; of graphs 12A and 12C, 2.5 times the probable error, and of graphs 12B and 12C, 15 times the probable error. This shows that there is a distinct difference between the original and mutant 1 in respect to the number of longitudinal septa; no distinct difference between the original and mutant 2, and a wide difference between mutant 1 and mutant 2 in this respect.

Culture No. 15.

Original. Plate 9. Graph No. 16A.			Mutant 1. Plate 9. Graph No. 16C.		
M.	1.08	.04	M.	1.34	.05
σ.	.90	.03	σ.	.95	.03
C.V.	84.29	4.42	C.V.	71.35	3.41

The difference between the means of graphs 16A and 16C is 5 times the probable error which is not considered large enough to show that there is any distinct difference between the original and the mutant in respect to the number of longitudinal septa in the conidia.

Color of Conidia

Culture No. 1.

The conidia of the original culture are quite dark with

a yellowish tint; those of mutant 1 are lighter and more yellow; the conidia of mutant 15 are darker than those of mutant 1, but not as dark as those of the original culture, and are without the yellowish tint.

Culture No. 7.

The original and mutant 1 have dark-brown conidia, so similar in color that no difference is discernible.

Culture No. 10.

Conidia of the original culture are light-brown, showing dark-brown in mass. Mutant 1 has light yellowish conidia, much lighter than those of the original, but appearing similar in mass. The conidia of mutant 2 are darker than those of the original and of mutant 1, and appear nearly black in mass.

Culture No. 15.

The conidia of the original culture are a light yellowish-gray, while those of the mutant are darker with a brownish cast.

Conidial Production

Culture Nol 1.

In mutant 1 of this culture numerous conidia are produced; slightly fewer in mutant 15, and a smaller number in the original than in either of the mutants.

Culture No. 7.

The original strain and mutant 1 both produce conidia abundantly in culture, but mutant 1 produces a great many more than the original, since it has more conidiophores and more conidia on each.

Culture No. 10.

Mutants 1 and 2 produce conidia in greater abundance than the original strain. Mutant 1 produces more conidia than mutant 2.

Culture No. 15.

The original culture produces very few conidia and as a rule they are formed either singly or in twos or threes on a conidiophore. Mutant 1 produces conidia abundantly in long chains.

Permanency of Mutants

Mutants which have been isolated from distinct sectors of the parent colony have usually remained constant in character. All of the mutants listed here have been carried through from twenty to fifty generations and have remained constant. A peculiarity exhibited by mutant 15 of culture No. 1 is that it seems frequently to undergo mutation and produce sectors which when isolated and studied are indistinguishable from mutant 1 of the same culture. The mutants from mutant 15 remain constant and show no further signs of mutation or reversion.

Frequency of Mutation

From these studies it has not been possible to form any definite opinion as to the frequency of mutation in Alternaria. In culture No. 1 mutation is of very frequent occurrence, sometimes one or more mutants appear in nearly every generation, again several generations may occur before another mutant is found. This culture has been under study for 14 months and has been gim-

ing rise to mutants rather constantly during that time. The majority of these mutants seem to be similar to each other, or so nearly similar that my contrast studies have failed to note differences. In culture No. 7 mutation seems to be rather infrequent, for the culture has been grown through ten generations since the appearance of mutant 1 without the appearance of a second mutant. Mutation is also of infrequent occurrence in culture No. 15, it having been under supervision for more than 30 generations with but a single mutation. In culture No. 10 mutation is of more frequent occurrence than in either culture No. 7 or No. 15 but is not of as frequent occurrence as in culture No. 1. The mutants of this culture, with the exception of mutant 2, have all been so similar to mutant 1 that no distinction has been possible.

VI. Conclusions

The foregoing contrast studies have shown that cultures isolated from variant sectors of strains of Alternaria grown from single spores differ from the original strain in a number of morphological characters. These differences are most striking in color of colonies as grown on agar-plates; in conidial length; presence of aerial mycelium; zonation of colonies; conidial production, and number of longitudinal septa in the conidia. Differences which are less common are those of color of the mycelium, color of the conidia, width of conidia and number of cross septations in the conidia. These mutants retain such characteristic differences through many generations without reverting to the original form. Mutation occurs, as a rule, in only one direction. Mutants tend

to form a darker colony, to have a more prominent zonation, to have less aerial mycelium and to produce more conidia than the original strain. It is noticable that where there is a decrease in color of the mycelium there is an increase in color of the conidia.

Mutation takes place in the cells of the mycelium rather than in the conidia and occurs in some strains and not in others. No attempt is made to explain the cause of mutation or to account for its occurrence in some strains and not in others. Since mutation occurs very frequently in Alternaria growing in culture it may be assumed that it also occurs in nature, though no means of proof are yet known.

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VIII. Explantation of Plates

Plate I. Frequency curves of conidial lengths.

Graph 1A. Original of culture No. 1.
Graph 1B. Mutant 1 of culture No. 1.
Graph 1C. Mutant 15 of culture No. 1.
Graph 5A. Original of culture No. 7.
Graph 5B. Mutant 1 of culture No. 7.
Graph 9A. Original of culture No. 10.
Graph 9B. Mutant 1 of culture No. 10.
Graph 9C. Mutant 2 of culture No. 10.
Graph 13A. Original of culture No. 15.
Graph 13B. Mutant 1 of culture No. 15.

Plate II. Frequency polygons of conidial widths of original and mutants of culture No. 1.

Graph 2A. Original.
Graph 2B. Mutant 1. Graph 2C. Mutant 15.

Plate III. Frequency polygons of number of cross septa in conidia of the original and mutants of culture No. 1.

Graph 3A. Original.
Graph 3B. Mutant 1.
Graph 3C. Mutant 15.

Plate IV. Frequency polygons of number of longitudinal septa in conidia of the original and mutants of culture No. 1.

Graph 4A. Original.
Graph 4B. Mutant 1.
Graph 4C. Mutant 15.

Plate V. Frequency polygons of width of conidia and number of cross and longitudinal septa in conidia of culture No. 7.

Graph 6A. Conidial width of original.
Graph 6B. Conidial width of mutant 1.
Graph 7A, Conidial cross septa of original.
Graph 7B. Conidial cross septa of mutant 1.
Graph 8A. Longitudinal septa of conidia in original
Graph 8B. Longitudinal septa of conidia in mutant 1

Plate VI. Frequency polygons of conidial width of mutants and original of culture No. 10.

Graph 10A. Original.
Graph 10B. Mutant 1.
Graph 10C. Mutant 2.

Plate VII. Frequency polygons of number of cross septa in original and mutants of culture No. 10.

Graph 11A. Original.
Graph 11B. Mutant 1.
Graph 11C. Mutant 2.

Plate VIII. Frequency polygons of number of longitudinal septa in conidia of original and mutants of culture No. 10.

Graph 12A. Original.
Graph 12B. Mutant 1.
Graph 12C. Mutant 2.

Plate IX. Frequency polygons of width of conidia and number of cross and longitudinal septa in the conidia of the original and mutant of culture No.15.

Graph 14A. Conidial width of original.
Graph 14B. Conidial width of mutant 1.
Graph 15A. Cross septa of original.
Graph 15B. Cross septa of mutant 1.
Graph 16A. Longitudinal septa of conidia in original.
Graph 16B. Longitudinal septa of conidia in mutant 1.

Plate X. Colonies of original and mutant 1 of culture 10 growing together in the same plate; showing the difference in color of colony and amount of aerial mycelium.

Plate XI. Colonies of original and mutant 1 of culture 1; showing difference in color of colonies and a variant sector in the original.

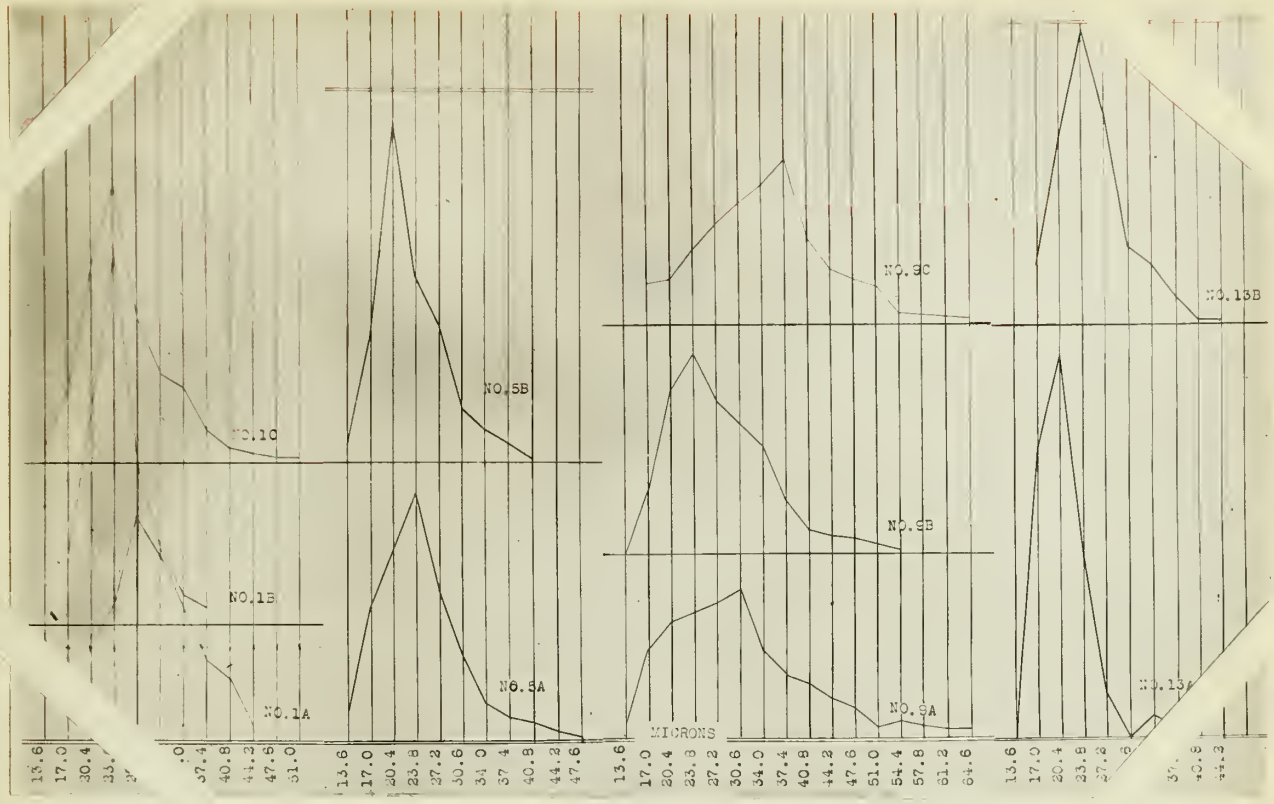
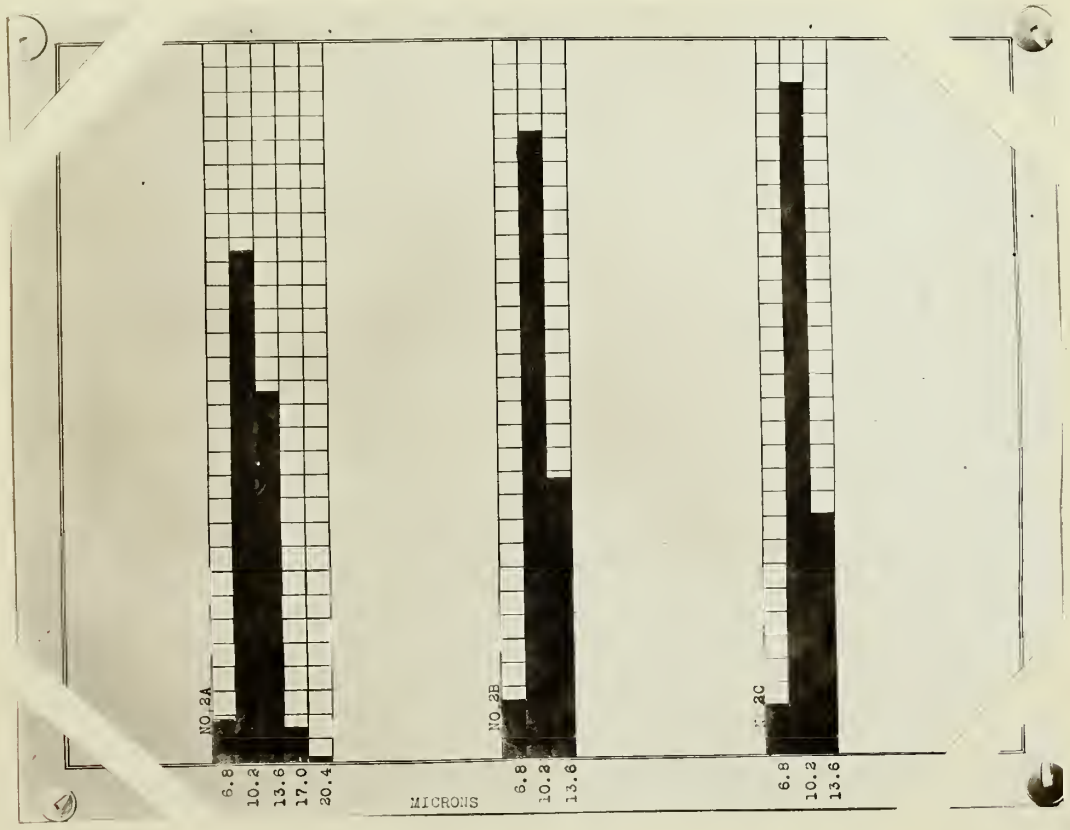


PLATE I.



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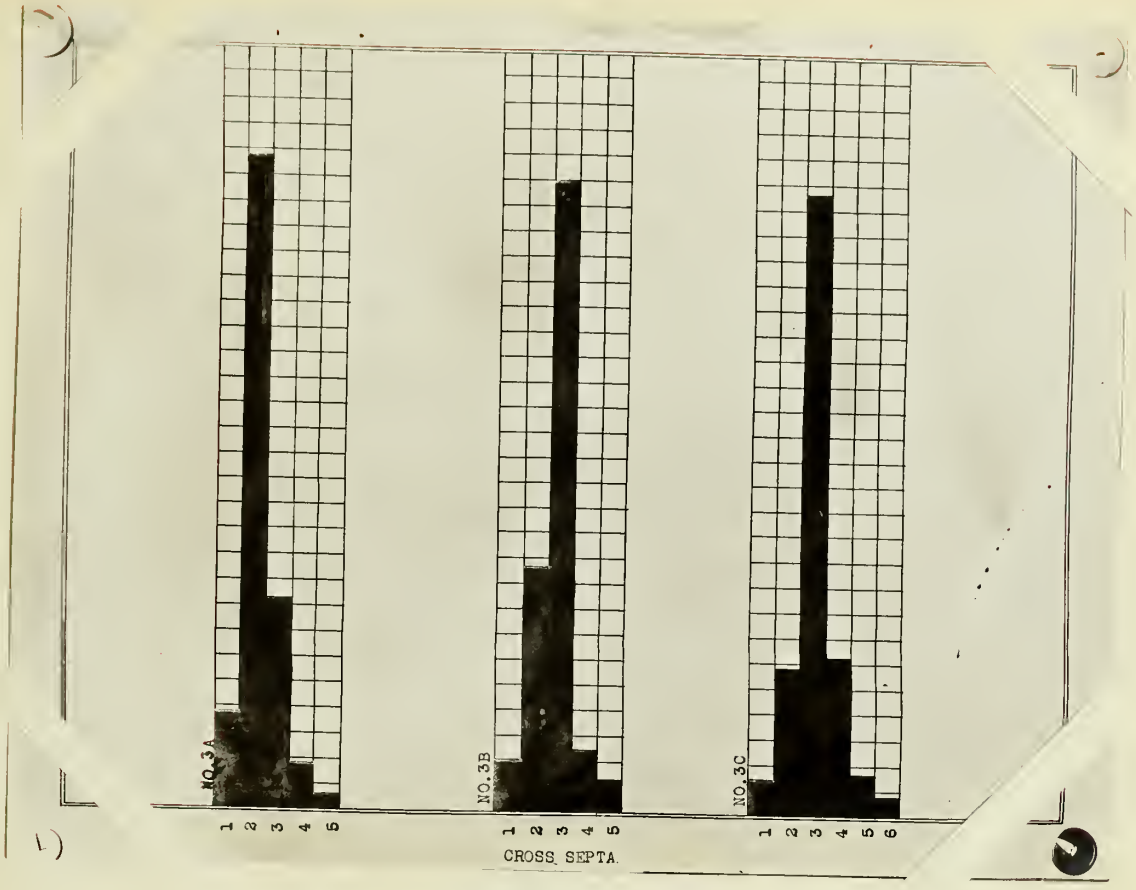

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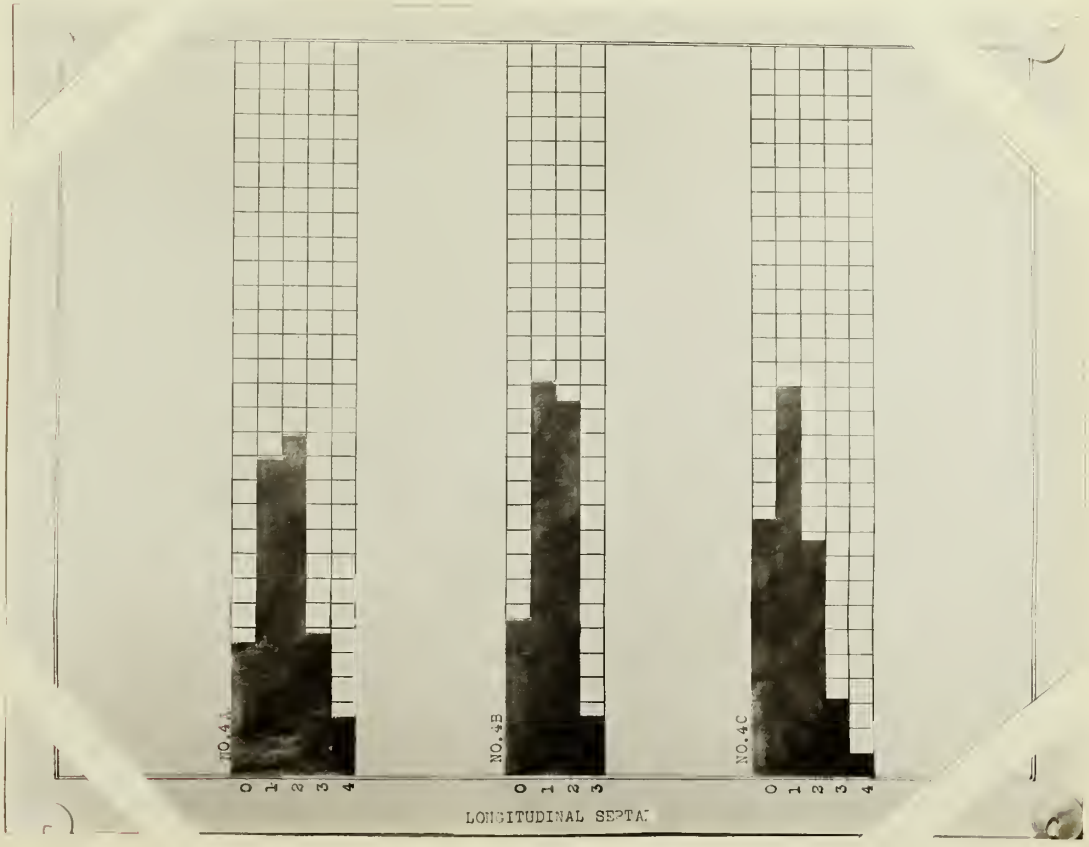

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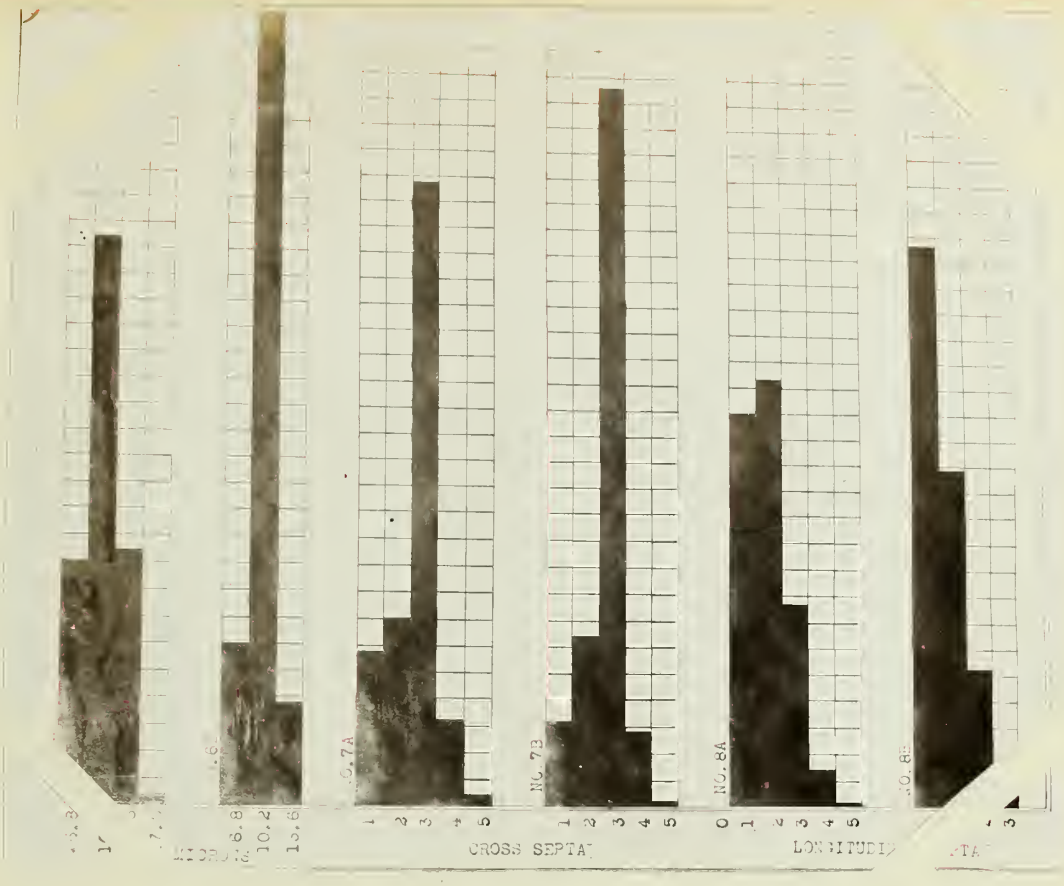

PLATE V.

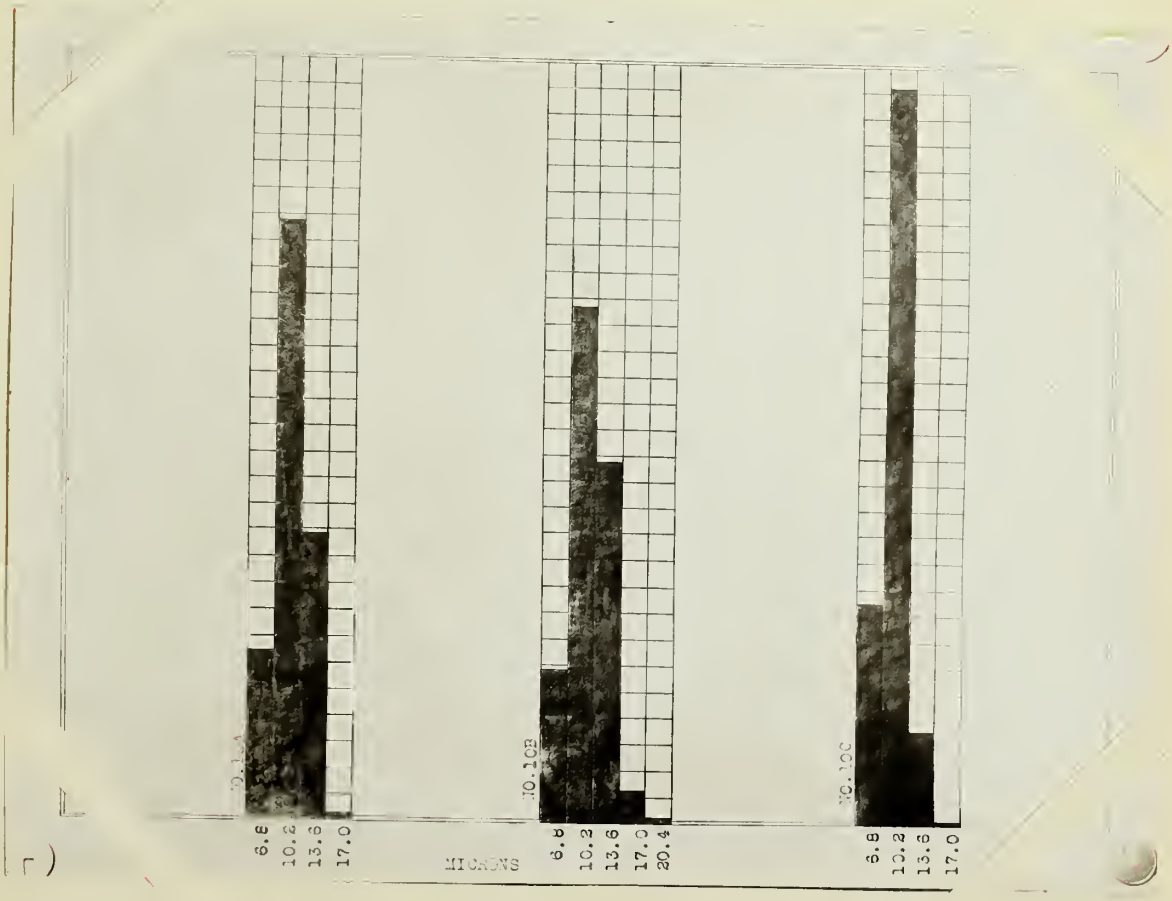

PLATE VI.

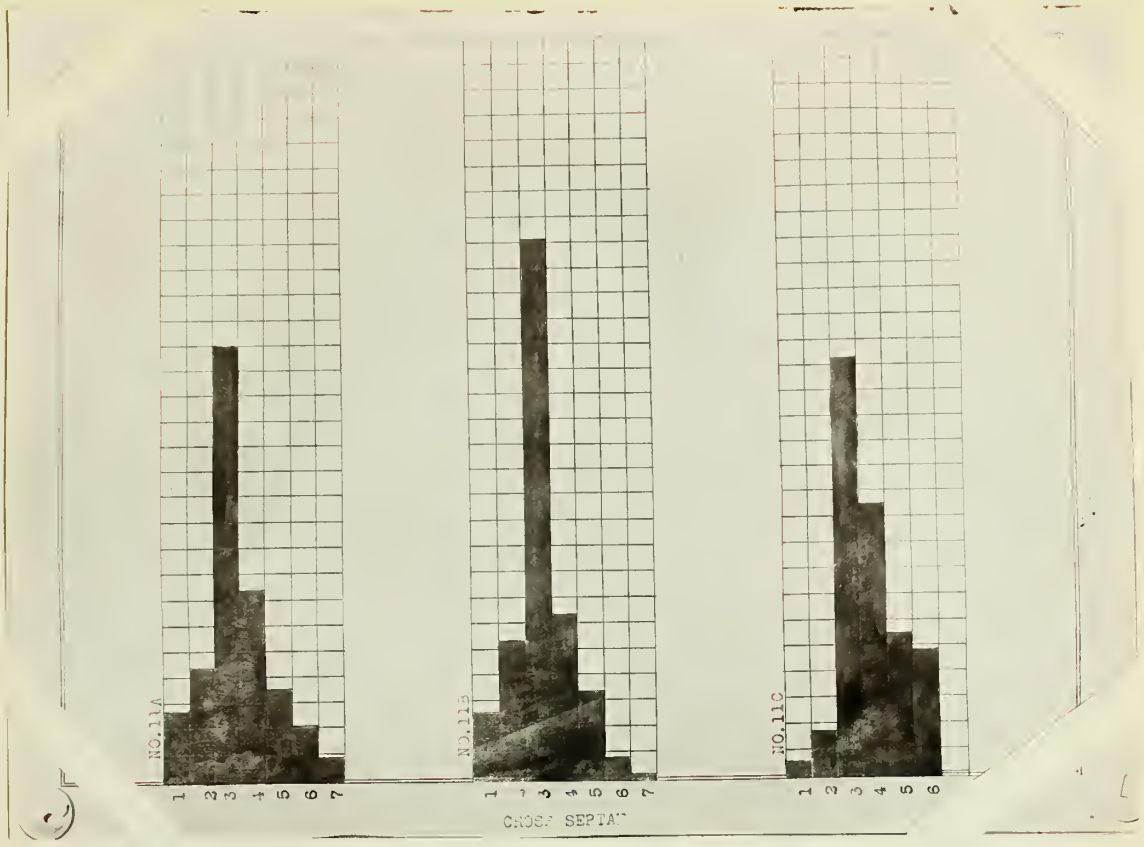

PLATE VII.

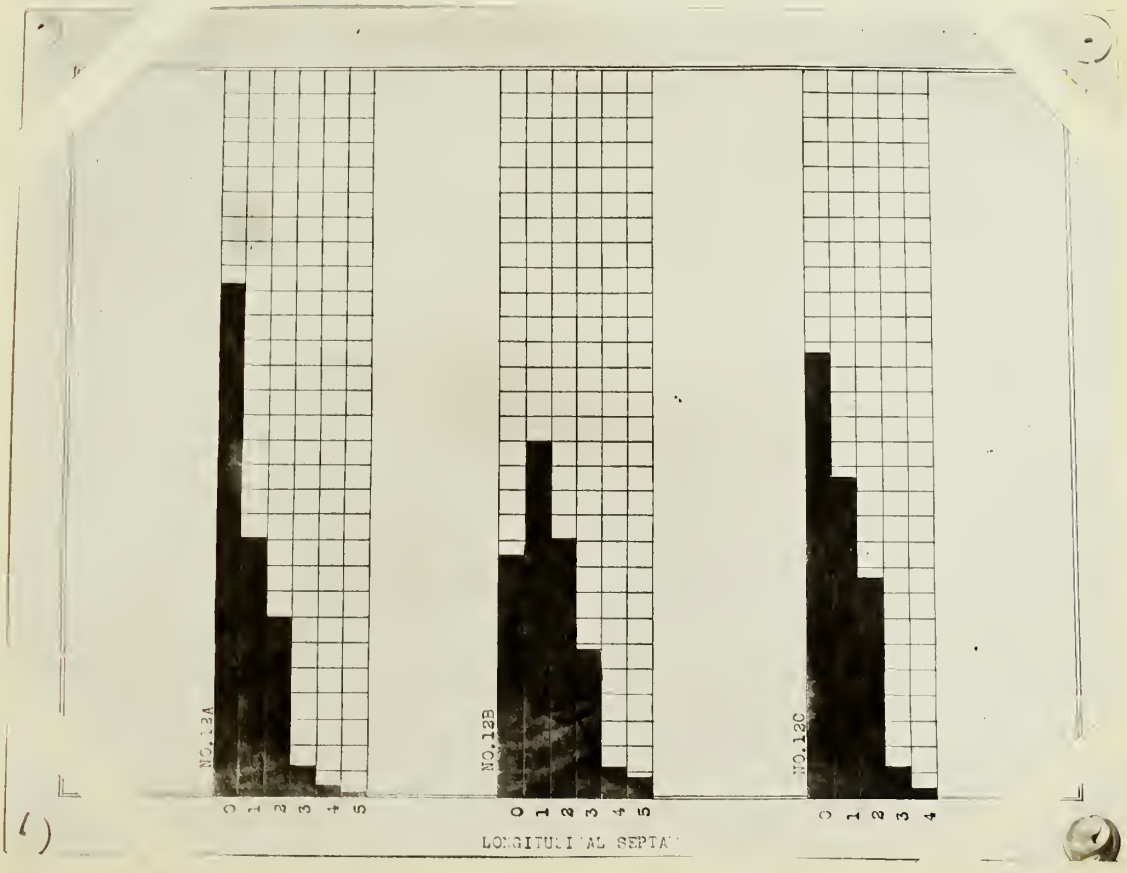

PLATE VIII.

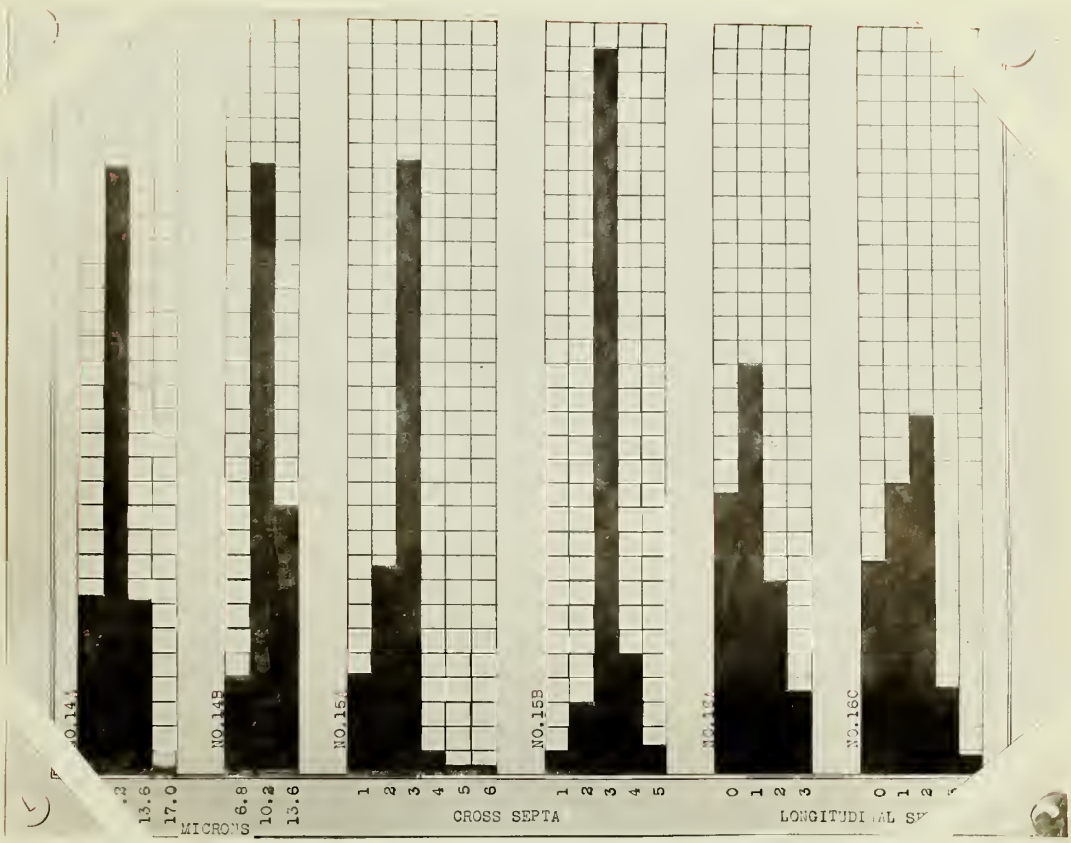
13.6
17.0
MICRONS
6.8
10.2
13.6
NO.15A
1 2 3 4 5 6
NO.15B
1 2 3 4 5
0 1 2 3
0 1 2
CROSS SEPTA

PLATE IX.

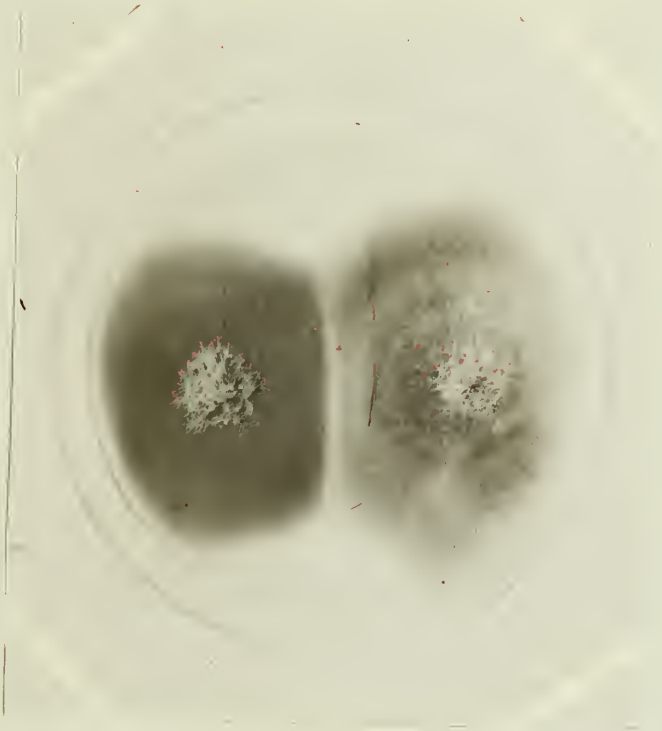

PLATE X.

PLATE XI.

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